A HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDY OF CHOLECYSTITIS

SUBMITTED FOR

M.D. IN PATHOLOGY

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY

DEPARTMENT OF PATHOLOGY

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Dr.P.Anupama

CERTIFICATE

This is certify that the dissertation work entitled "**A HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDY OF CHOLECYSTITIS"** submitted by **Dr.P.Anupama** is the work done by her during the period of study in this department from June 2005 to March 2008. This work has been done under my direct supervision and guidance.

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INTRODUCTION

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Gallstones are a major cause of morbidity throughout the world, necessitating hospitalization and cholecystectomy. Gallbladder is known to play crucial role in the formation of gallstones. Therefore, understanding the interaction between gallbladder mucosa and bile is an important step towards understanding the pathogenesis of gallstone disease.

Cholesterol saturated 'lithogenic' bile, originating from the liver and considered an important factor in gallstone formation, has been found in healthy individuals[1]. Lithogenic bile therefore cannot be the only factor involved in the process. Other factors such as supersaturation of bile with calcium[2], gallbladder mucus, prostaglandins and functional failure of electrolyte absorption by gallbladder mucosa also may influence gallstone formation.

Biliary calcium can reduce the solubility of cholesterol[3] rendering the bile lithogenic. Apart from being a critical initiating factor, calcium is physically incorporated into gallstones as well.

Gallbladder mucus has long been recognized as an important factor contributing to gall stone development[4, 5]. The implication of mucin in gallstone formation has been widely studied. Hypersecretion of mucus occurs during gallstone formation in humans [6,7,8,9,] and experimental animals[10]. Apart from forming the nucleus for calculus, the mucins form a structural component of gallstones as shown by histochemical studies on calculi[11, 12]. Calcium and prostaglandins can stimulate mucus secretion by gallbladder mucosa [13,14,15]

Normal human gallbladder contains predominantly sulphated acid mucin[16]. It is this sulphated mucin content that is increased in gallstone disease. Metaplastic and neoplastic gallbladder epithelium on the other hand shows an increase in sialomucins and decrease in sulphomucins. Several studies have suggested progression from metaplasia, through dysplasia, to adenocarcinoma of gallbladder[17]. The existence of such a pathway has not been definitely proven.

A study correlating gallbladder mucin histochemistry with morphology would help one understand the role of mucins in gallstone disease and carcinogenesis. Further, correlating the above with the chemical composition of stones could lead to the identification of a high risk group, with possible therapeutic implications.

AIMS AND OBJECTIVES

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1. Qualitative and quantitative assessment of gallbladder mucins in chronic calculous cholecystitis.

2. Correlating the mucin histochemistry and morphology of gallbladder in chronic calculous cholecystitis with each other and with the chemical composition of gallstones.

REVIEW Of LITERATURE

REVIEW OF LITERATURE

The gallbladder is one of the most frequently received specimens in surgical pathology laboratory, on which the pathologist routinely documents the presence of gallstones and inflammation. The formation of gallstones is closely linked to bile and mucosal epithelial interaction[18].

Gallbladder is a pear shaped sac that lies attached to the posterior aspect of the right lobe of liver. It measures about 10 cms in length and 3-4 cms in width and is divided into fundus, body and neck. The wall of gallbladder consists of mucosa, muscularis propria and perimuscular connective tissue. There is no muscularis mucosae and sub mucosa. The mucosa is lined by columnar epithelium with lightly eosinophilic cytoplasm and basally located nuclei. Only the neck part has true glands that are tubuloalveolar mucous type [19]. Metaplasia is not seen in normal gallbladder, but is common in cholelithiasis and cholecystitis. Metaplasia can be gastric or intestinal in type. Gastric metaplasia in turn is either foveolar (gastric foveolar type surface epithelium) or antral (gastric antral type glands). Intestinal metaplasia is characterized by columnar cells of intestinal type interspersed with goblet cells[20]; Endocrine and Paneth cells may be present[20].

Rokitansky Aschoff sinuses represent herniation of mucosal epithelium into lamina propria, smooth muscle or perimuscular connective tissue and are a common feature of chronic cholecystitis[21.]

The gallbladder concentrates, stores and releases bile. Approximately 800 – 1000ml of bile flows daily into the gallbladder from the liver. 40 –70ml of bile can be stored in the gallbladder [22.]

The proposed pathogenesis of gallstones, based on human studies and experimental animal models, involves steps such as bile saturation, nucleation, precipitation of cholesterol monohydrate crystals and growth to stone sized aggregates. Gallstone containing bile is characterized by supersaturation with cholesterol and rapid in vitro nucleation of cholesterol crystals[23,24].

Gallstones vary considerably in chemical composition, the basic constituents being cholesterol, calcium bilurubinate and calcium carbonate either alone or in combination [25.]

Figure 1. Multiple Small Cholesterol Gallstones

Cholesterol gallstones (75%-80%) include, pure cholesterol stones (10%) and mixed stones (10%). Pure cholesterol stones are yellow white, round to ovoid and smooth; have a crystalline or a laminated cut surface and measures up to 4cms in diameter. Mixed stones are those which have lower cholesterol content. Depending on the proportion of calcium carbonate, bilirubin and phosphates, mixed stones are laminated grey – white to black. They are usually smaller, multiple and faceted, may have laminated cut surface and a dark core. More than 80% of cholesterol stones lack calcium carbonate and are hence radiolucent .

Figure 2. Pigment Stones

Pigment stones (15%-25%) include brown and black stones. They are more common in Asia. By definition, they contain <25% - 30% cholesterol. They form as a result of increased unconjugated bilirubin in the bile, which then forms insoluble calcium salts. Black stones are black or deep brown, relatively small resist crushing, have an irregular shiny surface and on fracturing have a glass like, featureless appearance. They arise in sterile bile. Many black stones (50%-75%) contain enough calcium carbonates and phosphates to render them radio opaque. Brown stones are much softer than black ones, and have a rough, flaky appearance, and at times may be greasy appearing. They form in the context of biliary stasis and infection. Brown stones are usually radiolucent [26,27].

Before the appearance of gallstones there is always the formation of "biliary sludge" containing mucus gel, hydrophobic bile pigments, cholesterol, lecithin lipid crystals and cholesterol monohydrate crystals. The cholesterol crystal nucleation seems to occur in the mucus gel on the epithelial surface.

Mucin secretion by gallbladder and formation of biliary sludge (of which mucus gel is a crucial component) are important factors in the pathogenesis of gallstone disease and its sequelae [28,29,30,.]

MUCINS

Mucins are the chemical components of the secretion delivered by certain types of epithelial and connective tissue cells. The original term mucin was coined by Carpenter as early as in the year 1846 [31.] Reid and Clamp in 1978 suggested glycoconjugates as general term, which could be subdivided into 'proteoglycans' and 'glycoproteins' [32.]

STRUCTURE OF MUCIN:

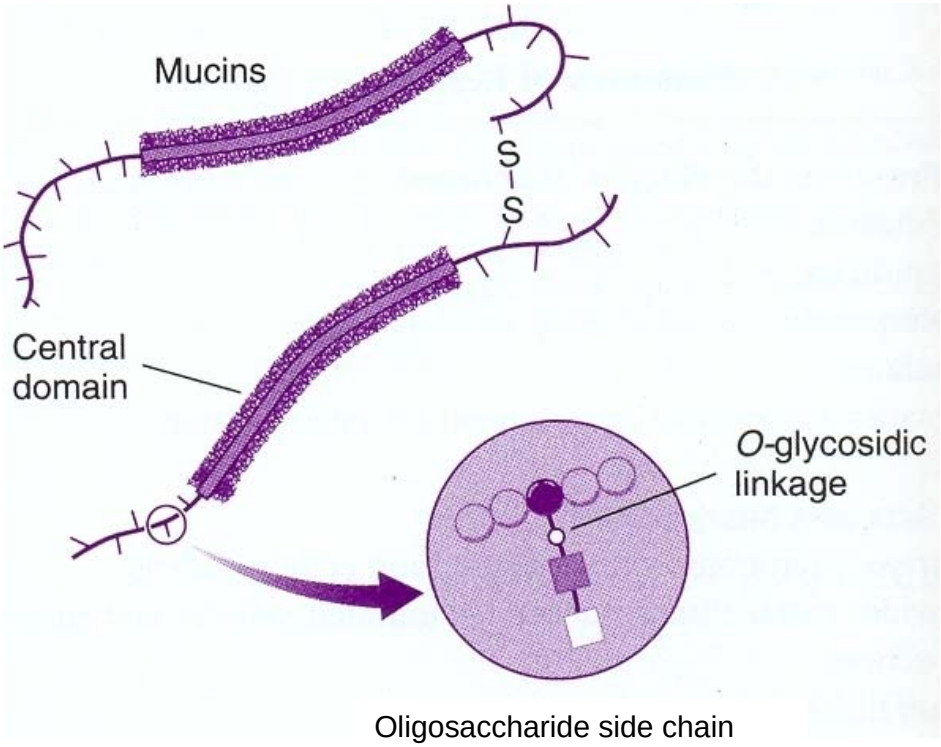


Figure 3. Structure of Mucin

Mucin glycoproteins are large, complex molecules consisting of a peptide backbone and numerous oligosaccharide side chains, which represents the products of mucin genes and glycols transferase genes respectively. Mucin molecules are among the largest molecule in nature, ranging in size from 3 - 23 million Daltons. Electron microscopy reveals them to be linear, flexible threads.

Free hexose groups are often available, together with certain acidic moieties, the presence of which will markedly influence histochemical reactivity. The different mucins may be present as a single type within a given tissue unit, or more usually as a mixture of different types. The synthesis of mucin is initiated in the rough endoplasmic reticulum of the producing cells and is completed in the golgi apparatus. Sulphation of the hexosamine molecule occurs in the golgi region [8,33.]

Scheme of an easy method to classify the mucin like a proteins the only two classical techniques.[8]

	AB-PAS	**HID-AB**
Neutral mucins	Red	Negative
Sulphomucins	Red-blue	Brown-black
Sialomucins	Red-blue	Blue
Sulpho-sialomucins	Red-blue	Brown-blue

AB-PAS, sequential staining with AB(2.5) (blue) and PAS (red); HID-AB, sequential staining with HID(brown to black) and AB(2.5) (blue).

The different types of mucins which can be distinguished histochemically are as follows [34]:

ACID MUCINS

1. Strongly sulphated
    a. connective tissue
    b. epithelial
2. Weakly sulphated

    Sulphated; histochemically atypical
3. Carboxylated; sialomucin
    a. enzyme – labile (N-acetyl form)
    b. enzyme – Resistant (N-acetyl O-acetyl form)
4. Sulfated sialomucin
5. Carboxylated; nonsulphated uronic acid (Hyaluronic acid)

NEUTRAL MUCINS

There are no subdivisions to this group

STUDIES ON MUCINS IN GALL STONE DISEASE

Role of gallbladder mucin as a nucleating factor and as a component of calculi and alterations in these mucins in non-neoplastic, preneoplastic and neoplastic diseases of the gallbladder have received wide attention among workers. Review of literature in this field revealed many studies connecting mucins with gallstone diseases, which could be grouped into sections as shown below:

1) Biochemical studies on gallbladder bile.
2) Studies on gallstones.
3) Mucin histochemistry of non neoplastic gallbladder mucosa
4) Mucin histochemistry of neoplastic gallbladder mucosa
5) Electron microscopic molecular and animal experimental studies.

BIOCHEMICAL STUDIES ON BILE:

The major acid mucins secreted by gallbladder mucosa are sulphomucin [35].Increase in the mucin content of bile in patients with gallstone disease, as against controls has been shown by many workers using biochemical techniques [9,35,36].

Harvey et al however found no difference in the mucus content of bile, between cases with and without gallstones. Their study consisted of isolating gallbladder mucus by sepharose gel filtration followed by ultracentrifugation [37].

Levy et al studied model bile and demonstrated the accelerating effect of mucins on nucleation of cholesterol monohydrate crystals, an early step in lithogenesis [30].

STUDIES ON GALLSTONES:

Womack et al as early as in the year 1963, demonstrated the presence of mucopolysaccharides in gallstones. Whole stones were sectioned and stained for mucins [11].

Subsequently other workers also showed presence of mucin as a structural component of cholesterol stone matrix [38] . The amount of mucins in cholesterol stones was found to be less, compared to pigment stones . It was also shown that the mucins in pigment stones are mostly sulphated. The bridging action of sulphomucins promoting solidification of the mucus gel during stone formation was suggested [12].

MUCIN HISTOCHEMISTRY OF NON NEOPLASTIC GALLBLADDER MUCOSA:

Normal gallbladder epithelium contains sulphated acid mucins[39], with very small quantities of nonsulphated mucins. In recent years there has been widespread use of mucin histochemistry as an aid to the diagnosis of diseases. Particular attention has been paid to alterations in the relative amounts of sulphated and nonsulphated mucins present within epithelial cells. Many techniques are available for this purpose; the most widely used being the High Iron Diamine – Alcian Blue (HID-AB) technique.

Qualitative changes in mucin occur in metaplastic gallbladder mucosa[16,19,20,40]. In cholecystitis without metaplasia no qualitative alterations in mucins have been observed, though the quantity of sulphomucin increases[16.] Presence of sulphated mucins in the surface epithelium and neck glands of gallbladder and nonsulphated acid mucins and neutral mucins in the goblet cells of intestinal metaplasia and in metaplastic gastric epithelium have been confirmed by studies on large number of cases [20]

Madrid et al studied epithelial mucins of gallbladder using conventional techniques and demonstrated the presence of sulphated and carboxylated mucins, the former predominating [40.] Further localization of sulphomucins to the surface epithelium, sialomucins to the mid portion and either sulphomucins or PAS positive, probably neutral, mucins to the deeper glands was demonstrated in one study [41]

MUCIN HISTOCHEMISTRY AND NEOPLASTIC GALLBLADDER MUCOSA:

The precise relationship between gallbladder cancer and its precursors remain ill defined. Several studies have shown that invasive carcinoma is preceded by dysplasia, but progression from cholelithiasis to dysplasia has not been proven.

It has been suggested that antral metaplasia may be the pathogenetic link in this context [42,43,44,45]. There are also several studies that suggest a progression from intestinal metaplasia through dysplasia, to adenocarcinoma of the gallbladder [46,47,48,49] . These studies point out the increased incidence of intestinal metaplasia in specimens with carcinoma, presence of intestinal metaplasia in the vicinity of carcinoma and within the carcinoma[48] . Intestinal metaplasia therefore is considered to represent

a neoplastic transformation or a predisposition to neoplastic transformation.

The importance of extensive sampling in the detection of intestinal metaplasia was emphasized by Durate et al [49].

Histochemical and immunohistochemical studies have shown similarities between pyloric metaplasia, intestinal metaplasia and carcinoma of gallbladder. Presence of endocrine cells in pyloric metaplasia suggests that these glands are an integral component of intestinal metaplasia. It is therefore possible that intestinal and gastric metaplasia arise from stem endodermal cells that differentiate into either direction[43,44,45].

Immunohistochemical studies an normal, metaplastic and dysplastic gallbladder mucosa have shown a difference in mucin expression in these groups. Normal epithelium had mucin phenotypes similar to gastric pyloric mucosa (MUC5), while metaplastic and dysplastic epithelium expressed colonic/intestinal mucin phenotype (MUC2), [50].

ELECTRON MICROSCOPIC, MOLECULAR AND ANIMAL EXPERIMENTAL STUDIES:

Apart from HID-AB and PAS staining, electron microscopy[51,52], lectin histochemistry [40] and immunohistochemistry[41,42,53] have been applied to the study of mammalian gallbladder mucins. Lee induced different types of stones in experimental animals along with histochemical staining of epithelium for mucins and biochemical analysis of the mucins [28,29]. In this study comprising composition of stones with histochemical and biochemical analysis of mucins, no qualitative changes were observed, despite a quantitative increase in mucin.

Experimental studies have been performed on ground squirrel with application of bile biochemistry, mucosal morphology and mucin histochemistry [52,54,55,56]. Mucin hypersecretion was found to be an initiating event in gallstone formation. Histochemically, as in most other studies, sulphomucins predominated.

More sophisticated techniques such as mucin gene expression in cell lines however have shown on altered mucin core peptide in cholecystitis [57]. Mucin core proteins, it was found, altered with increasing degree of inflammation.

MATERIAL

MATERIAL

A total number of 40 specimens were selected from gallbladders with clinical and histopathological diagnosis of chronic calculous cholecystitis received in the department of pathology PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH during the period of 2005 to 2007. Criteria for selection were 1) Histopathological confirmation of chronic calculous cholecystitis. 2) Presence of calculi accompanying the specimen. 3) Availability of sufficient mucosa and well preserved lining epithelium in sections. 4) Availability of corresponding paraffin blocks. While 36 specimens fulfilled the above criteria, 4 cases had only biliary sand in the container. 3 gallbladders resected for choledochal cysts, were taken as controls.

METHODS

METHODS

Haematoxylin & Eosin stained sections of the selected specimens were screened and one to two sections with adequate amount of well preserved mucosa with lining epithelium were chosen. The corresponding paraffin blocks were selected and isolated. Four sections were cut on each of the selected blocks for special stains. A proforma was prepared for assessment as shown in Appendix I. The slides were assessed according to the proforma.

HAEMATOXYLIN & EOSIN STAIN

Sections stained with Haematoxylin & Eosin were assessed for the intensity of inflammation and degree of fibrosis, which in turn were graded as mild, moderate and severe (1+, 2+, 3+) (figures 4,5,6,7,8). The number of Rokitansky Aschoff sinuses was indicated as many, few and nil (figure 9). Gastric metaplasia and intestinal metaplasia were also noted and indicated as present or absent (figures 10,11).

MUCIN HISTOCHEMISTRY

The following special stains for mucin were done with a view to assess the quantity and quality of mucins in the superficial and deep parts of gallbladder mucosa.

1. High Iron Diamine – Alcian Blue Stain (HID-AB)
2. Alcian Blue – Periodic Acid Schiff (AB-PAS)

HIGH IRON DIAMINE – ALCIAN BLUE STAIN (HID-AB) [58]

REAGENTS:

N N dimethyl-meta –phenyline diamine dihydrochloride - 120 mg

N N dimethyl – para –phenyline diamine dihydrochloride - 20 mg

Distilled water - 50ml

Ferric chloride (60 % BDH SOLUTION) -1.4 ML

Dissolve the two diamine salts simultaneously in distilled water, add to the ferric chloride solution and mix.

PROCEDURE

1. Dewax the positive control section and the test sections and bring to distilled water.
2. Treat all the sections with diamine solutions for 24 hours.
3. Wash well in running water.
4. Counterstain with 1% AB (PH 2.5) in 3 % acetic acid for 5 minutes wash and counterstain with 0.5 % aqueous neutral red for 2-3 min wash in water.
5. Rinse in absolute alcohol.
6. Clear in xylene and mount.

RESULTS

Sulphated mucins – black/brown

Carboxylated mucins – blue

ALCIAN BLUE - PERIODIC ACID SCHIFF STAIN (AB-PAS) [59]

REAGENTS

Solution A

Alcian blue - 1gm

3 per cent Acetic acid – 100ml

Solution B

Periodic acid solution

Periodic acid - 1gm

Distilled water 200 ml

Solution C

Basic fuschin 1gm

Potassium metabisulphite 2gm

Concentrated hydrochloric acid 2ml

Activated charcoal 2gm

Distilled water 200ml

PROCEDURE

1. Dewax section and bring to water
2. Alcian blue solution, 5 min
3. Wash in water, then in distilled water
4. 1% aqueous periodic acid, 5 min
5. Rinse well in distilled water.
6. Schiff's reagent 15 min.
7. Wash in running tap water 5-10 min.
8. Stain nuclei lightly with haematoxylin solution.
9. Wash in water.
10. Rinse in absolute alcohol.
11. Clear in xylene and mount.

RESULTS

Acid mucins – blue

Neutral mucins – magenta

A scoring system was devised, based on the percentage positivity of cells in each field under low power examination (10X), as shown below:

75% -100 %- 5+

50% - 75% - 4+

25% - 50% - 3+

5% - 25% - 2+

0% - 5% - 1+

(Figures 12,13,14,15,16)

The values were tabulated and statistically assessed using ANOVA, t-test, χ^2 (Chi^2) test.

Biochemical analysis of calculi, wherever available (36 samples) was performed to determine the chemical composition of calculi. Gross parameters such as external appearance, colour, number of stones and weight were noted. The following biochemical procedures were carried out on the 36 samples of calculi.

BIOCHEMICAL ANALYSIS [60]

CHOLESTEROL

Wash the gallstones with water and dry. Powder the stone and heat some with successive small portions of ether in a test tube by inserting the tube in some warm water and filter. Dissolve a little of residue obtained on evaporation of the ether in chloroform and add a little of mixture of acetic anhydride and sulphuric acid (in the proportion of 10 ml to 0.1 ml). A dark green colour develops rapidly.

PHOSPHATE AND CALCIUM OXALATE

Treat the remaining residue after ether extraction, with dilute hydrochloric acid (25%). This dissolves the inorganic salts present. Filter and test the filtrate for phosphate with molybdate. Make some of the solution alkaline with ammonia and add acetic acid and ammonium oxalate solution. If calcium is present a precipitate of calcium oxalate is formed.

BILE PIGMENT

Test the precipitate remaining after treatment with hydrochloric acid for bile pigments. Wash the material remaining on the filter paper and extract with warm chloroform. Examine the chloroform extract for bilirubin by means of diazo reagent. Change to pink colour indicates presence of bile pigment.

The mucin histochemistry scores obtained were tabulated, separately for the three grades of inflammation and fibrosis. An attempt was made to correlate the score with degree of inflammation, fibrosis, presence of metaplasia (intestinal and pyloric) and type of stone. Statistical assessment of the results was also performed.

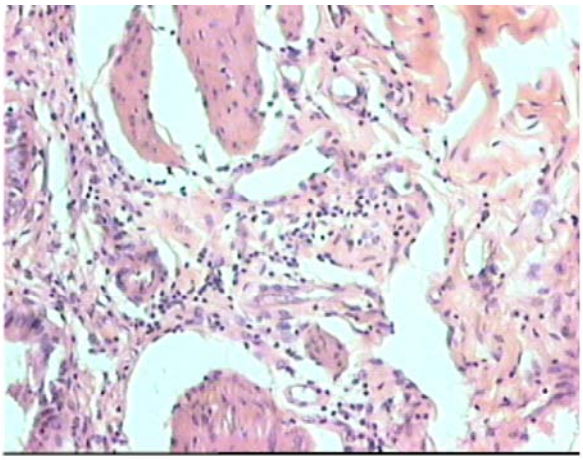


Figure 4. Mild (Grade I) Inflammation – Gall Bladder.
A few lymphocytes are seen between smooth
muscle bundles. H & E. (x 100)

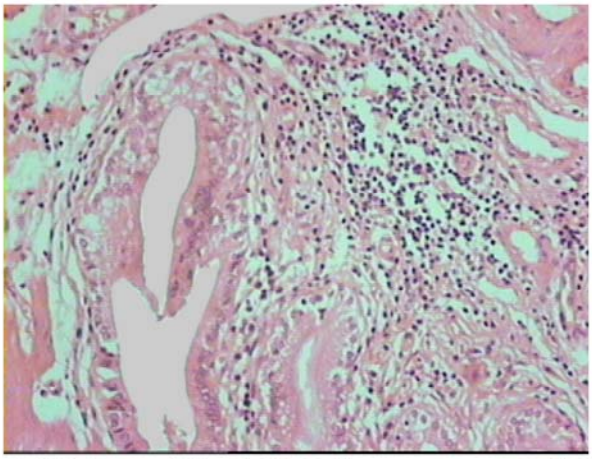


Figure 5. Moderate (Grade II) Inflammation – Gall Bladder.
Lymphocytes are seen in sheets over an area.
A Rokitansky – Aschoff Sinus is also present. H & E. (x 100)

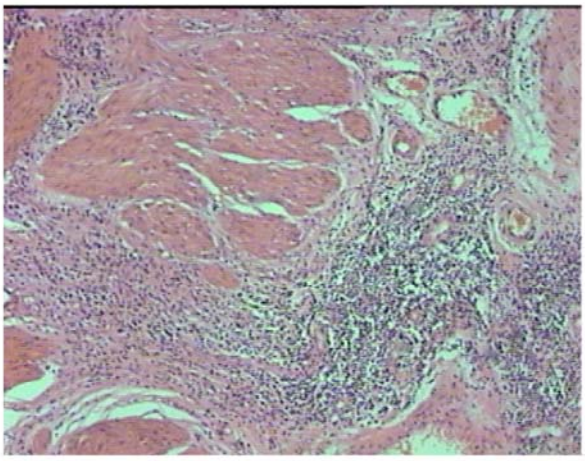


Figure 6. Severe (Grade III) Inflammation - Gall Bladder.
Dense Sheets of Lymphocytes extended between
smooth muscle bundles. H & E. (x 100)

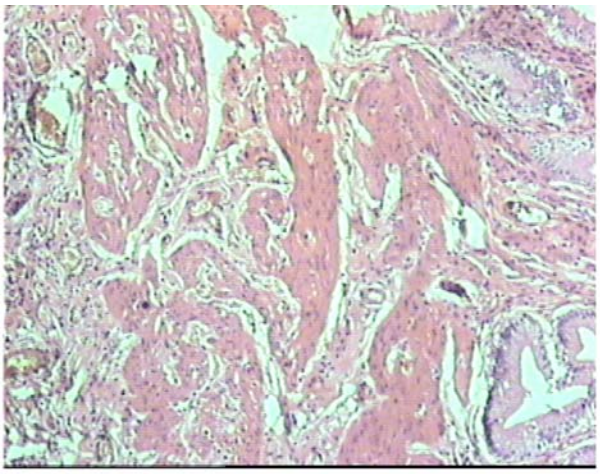


Figure 7. Moderate Fibrosis – Gall Bladder. H & E. (x 100)

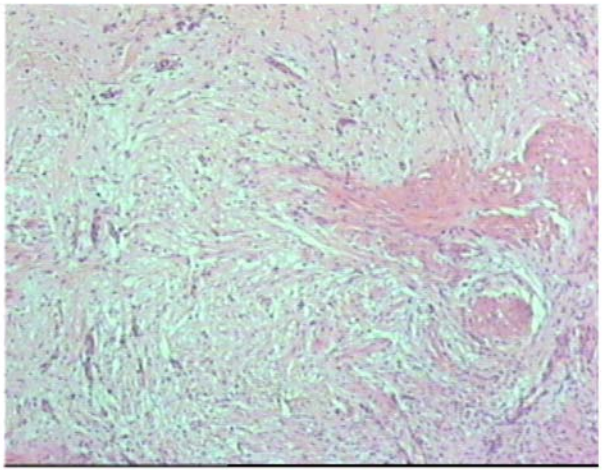


Figure 8. Severe Fibrosis – Gall Bladder.
H & E. (x 100)

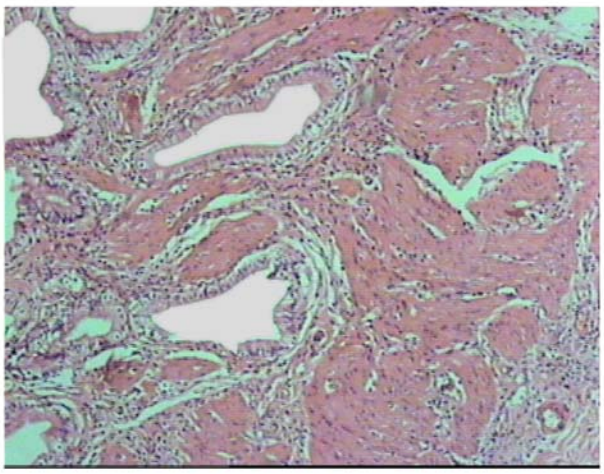


Figure 9. Photomicrograph shows Rokitansky Ascho ff Sinuses.
Mild inflammation also present. H & E. (x 100)

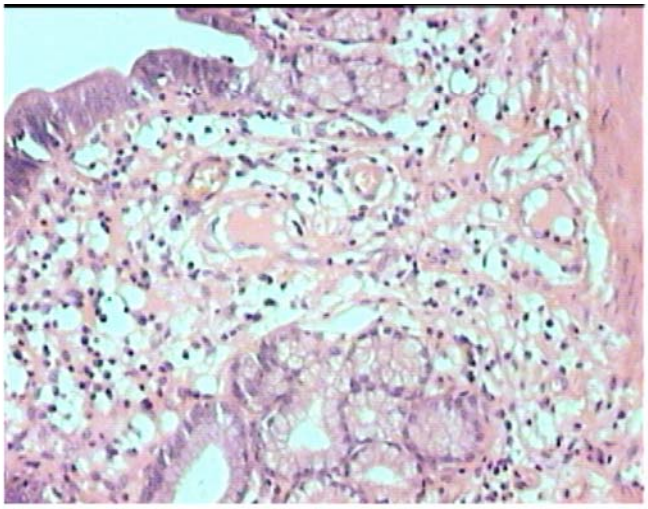


Figure 10. Photomicrograph shows Pyloric Metaplasia in the deeper mucosa. H & E. (x 100)

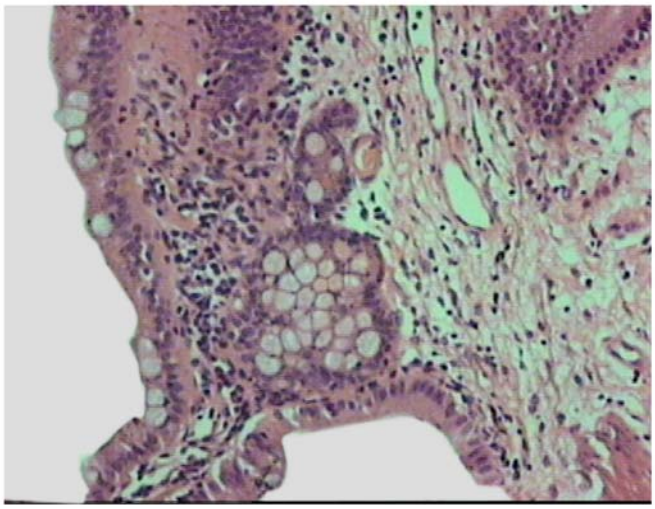


Figure 11. Intestinal Metaplasia characterised by Goblet cells in the Gall Bladder mucosa. H & E. (x 100)

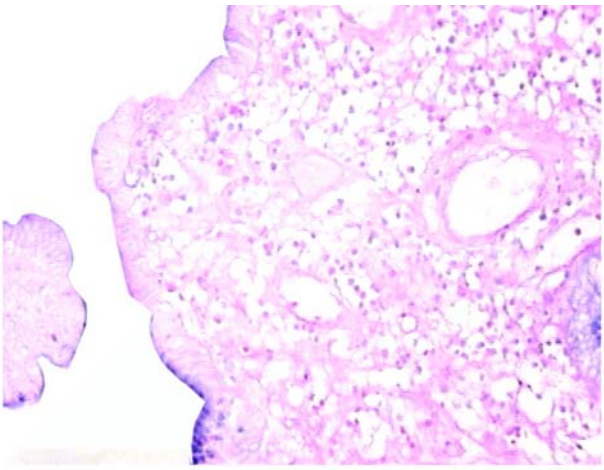


Figure 12. Score 1 - About 5% of cells contain Mucin AB-PAS(x100)

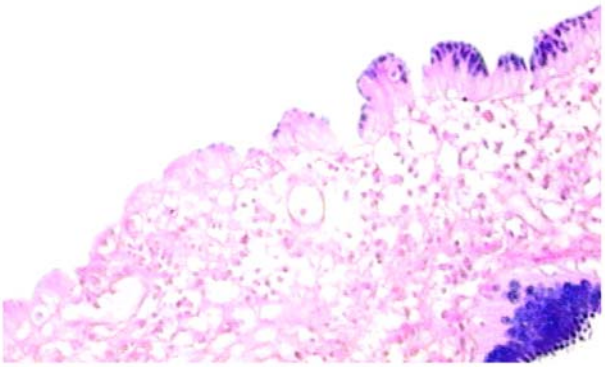


Figure 13. Score 2 - About 25% of cells contain Mucin AB-PAS(x100)

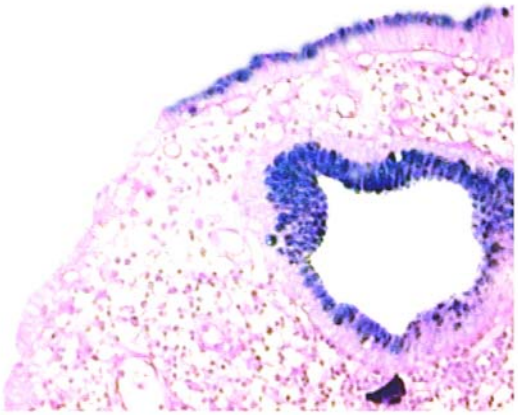


Figure 14. Score 3 - About 50% of cells contain Mucin AB-PAS(x100)

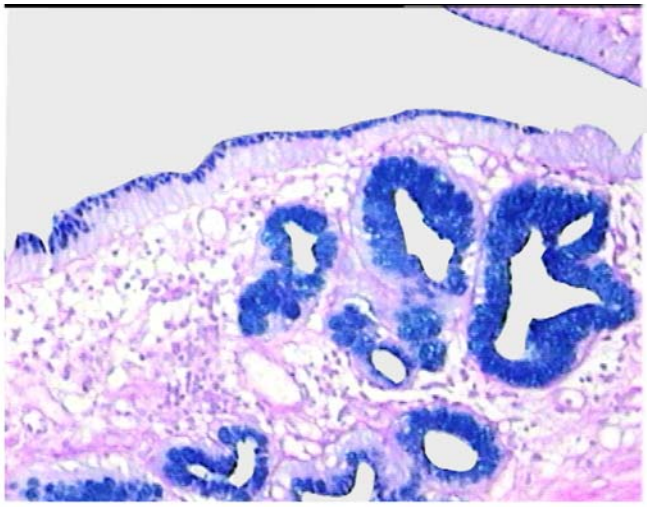


Figure 15. Score 4 - About 75% of cells contain Mucin
AB-PAS(x100)

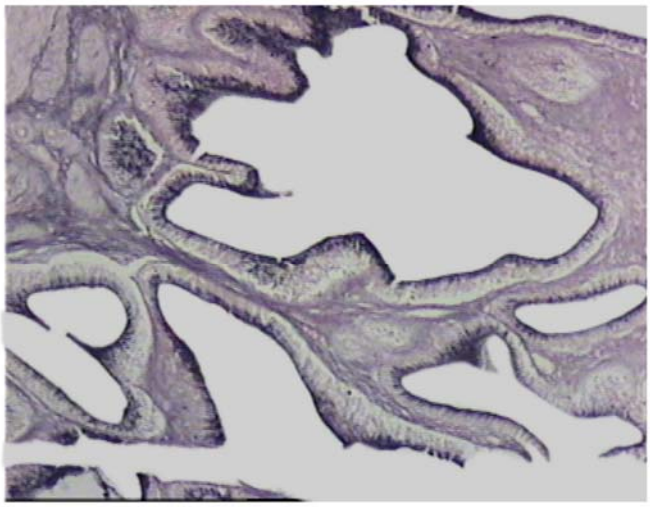


Figure 16. Score 5 - About 75-100% of cells contain Mucin
HID-AB(x100)

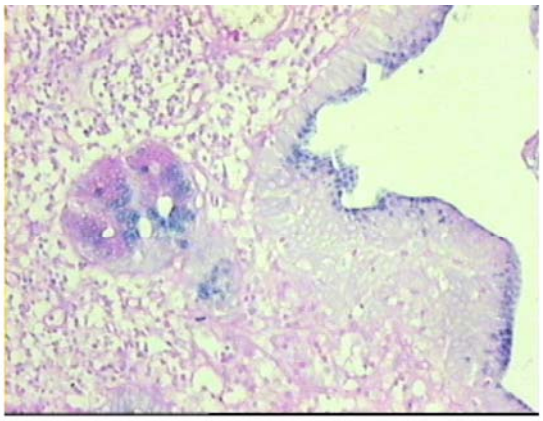


Figure 17. Gastric Metaplasia showing PAS and Alcian Blue positive Mucins. AB-PAS(x100)

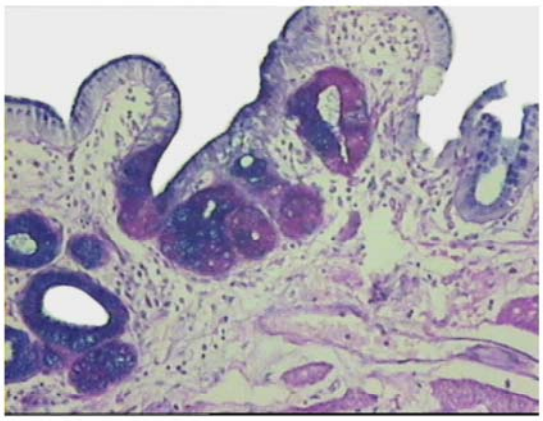


Figure 18. PAS positivity in areas of Gastric Metaplasia. AB-PAS(x100)

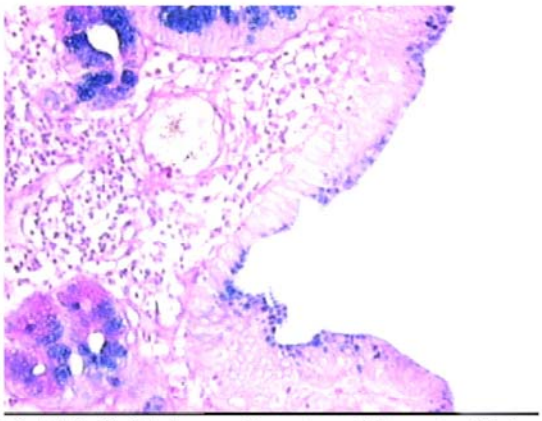


Figure 19. This field shows Gastric and Intestinal Metaplasia. The Gastric Metaplastic epithelium shows PAS and Alcian Blue Positive Mucins. Goblet cells of Intestinal Metaplasia are Alcian Blue positive Mucins. AB-PAS(x100)

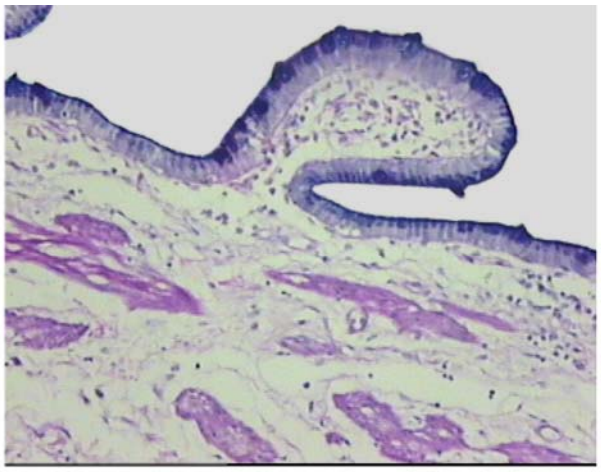


Figure 20. Intestinal Metaplasia – Gall Bladder
Goblet cells contain Alcian Blue positive mucin
AB-PAS (x 100).

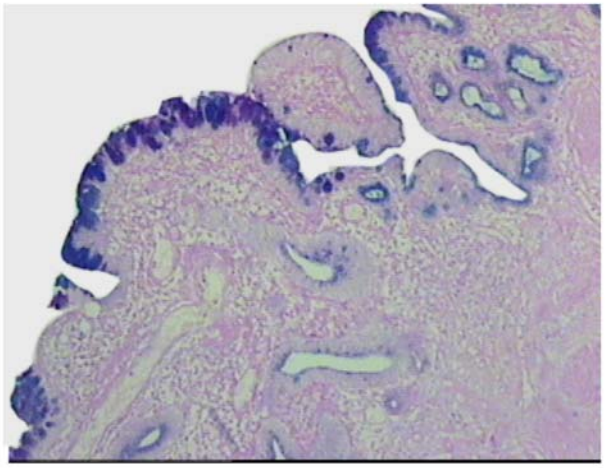


Figure 21. Intestinal Metaplasia – Gall Bladder
AB-PAS (x400)

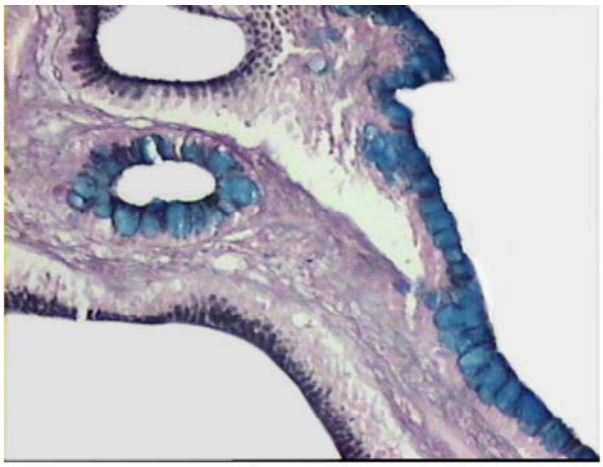


Figure 22.
Gastric Metaplasia – Gall Bladder
The Non – Neoplastic epithelium is HID positive (Brown)
Metaplastic epithelium Alcian Blue positive (Blue)
HID-AB (x400)

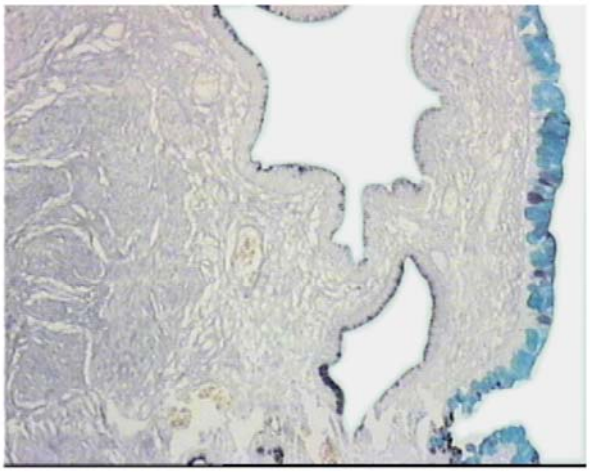


Figure 23. Gastric Metaplasia – Gall Bladder
Metaplastic epithelium is Alcian Blue positive
HID-AB (x100)

Figure 24. Intestinal Metaplasia – Gall Bladder
Goblet cells contain Alcian Blue positive mucin.
The columnar cells are HID positive (Brown)
HID-AB (x400)

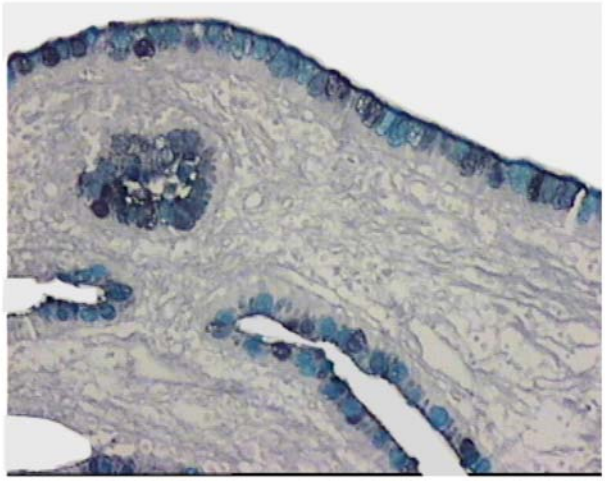


Figure 25. Intestinal Metaplasia – Gall Bladder HID-AB(x400)

RESULTS

RESULTS

The three specimens of gallbladder used as a controls showed predominantly Alcian Blue positive mucins in the surface and deep mucosal epithelium. PAS positivity was seen in traces.

HID –AB stain showed strong HID positivity indicating predominance of sulphomucins.(TABLE 1)

TABLE 1

Techniques		AB-PAS	HID-AB
		Superficial	Deep
AB-PAS	AB	4.7	2.6
	PAS	0.06	0.03
HID-AB	HID	4.5	2.8
	AB	0	0.03

INFLAMMATION AND MUCIN HISTOCHEMISTRY

Of the total 36 cases studied, 9 showed mild (Grade I) inflammation, 16 cases showed moderate (Grade II) inflammation and 11 cases showed severe (Grade III) inflammation. The mean scores for Alcian Blue, PAS and High Iron Diamine positive mucins in the three groups were tabulated. (Table 2-5)

TABLE 2

AB – PAS STAIN

INFLAMMATION GRADE	ALCIAN BLUE [ACID MUCIN] MEAN SCORE	
	SUPERFICIAL	DEEP
I (9 cases)	2.7	3.4
II (16 cases)	2.2	3.1
III (11 cases)	1.95	2.5

The mean scores for Alcian Blue positive (acid) mucins in the superficial and deep mucosa, in the three grades of inflammation are shown. There is progressive decrease in the mean scores for Alcian Blue positive (acid) mucins in the superficial and deep mucosal epithelium, with increasing grades of inflammation.

TABLE 3

AB –PAS STAIN

INFLAMMATION GRADE	PAS [NEUTRAL MUCIN] MEAN SCORE	
	SUPERFICIAL	DEEP
I (9 cases)	0.25	0.14
II (16 cases)	0.11	0.31
III (11 cases)	0.29	0.4

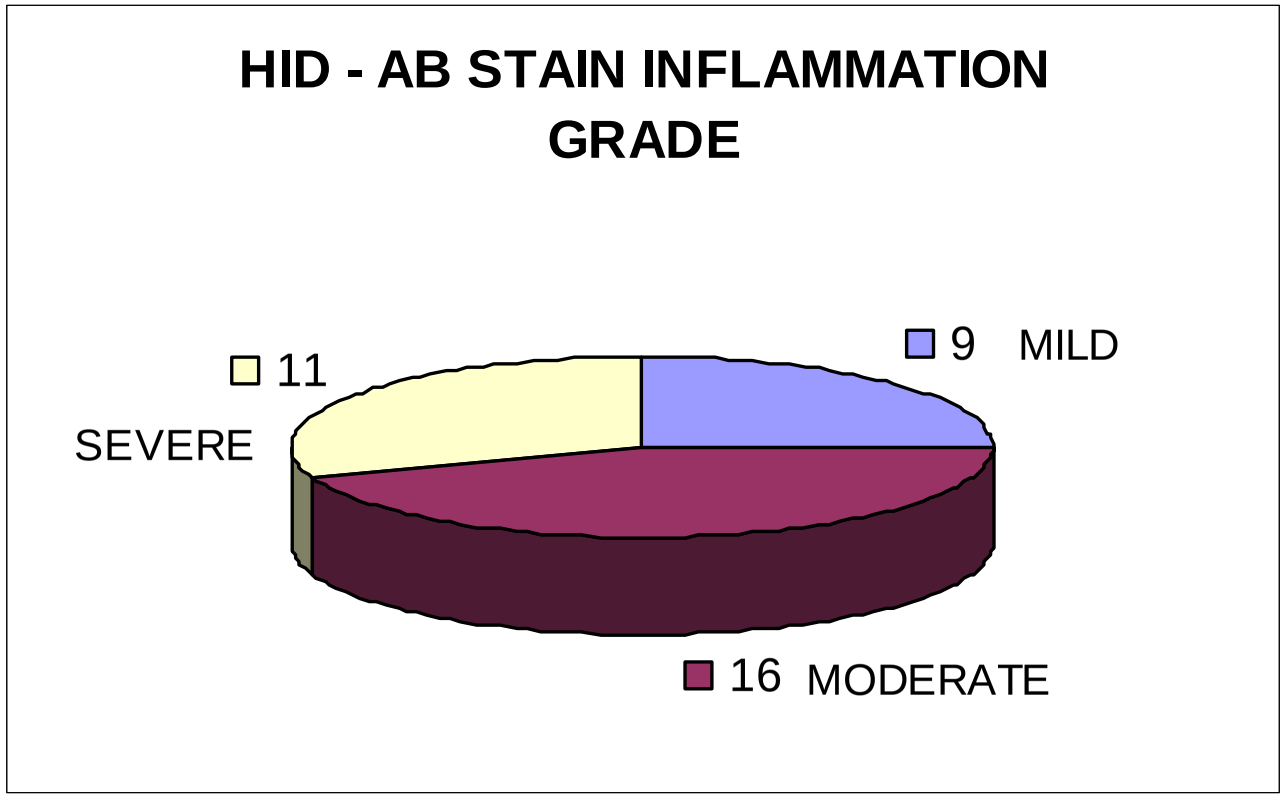
The mean scores for PAS positive mucins in the superficial and deep mucosa are tabulated against grades of inflammation. The scores are higher in Grade III inflammation, than in Grade 1 inflammation. Grade II inflammation however shows random scores , not conforming to any pattern.

TABLE 4

HID –AB STAIN

INFLAMMATION GRADE	HID – [SULPHOMUCIN] MEAN SCORE	
	SUPERFICIAL	DEEP
I (9 cases)	3.9	3.6
II (16 cases)	2.5	3.9
III (11 cases)	2.1	2.8

The mean scores for HID positive sulphomucins is tabulated against the three grades of inflammation.The scores are the lowest for Grade III inflammation. A progressive decrease in HID scores is obvious in the superficial mucosal epithelium.


HID - AB STAIN INFLAMMATION GRADE
9 MILD
11 SEVERE
16 MODERATE

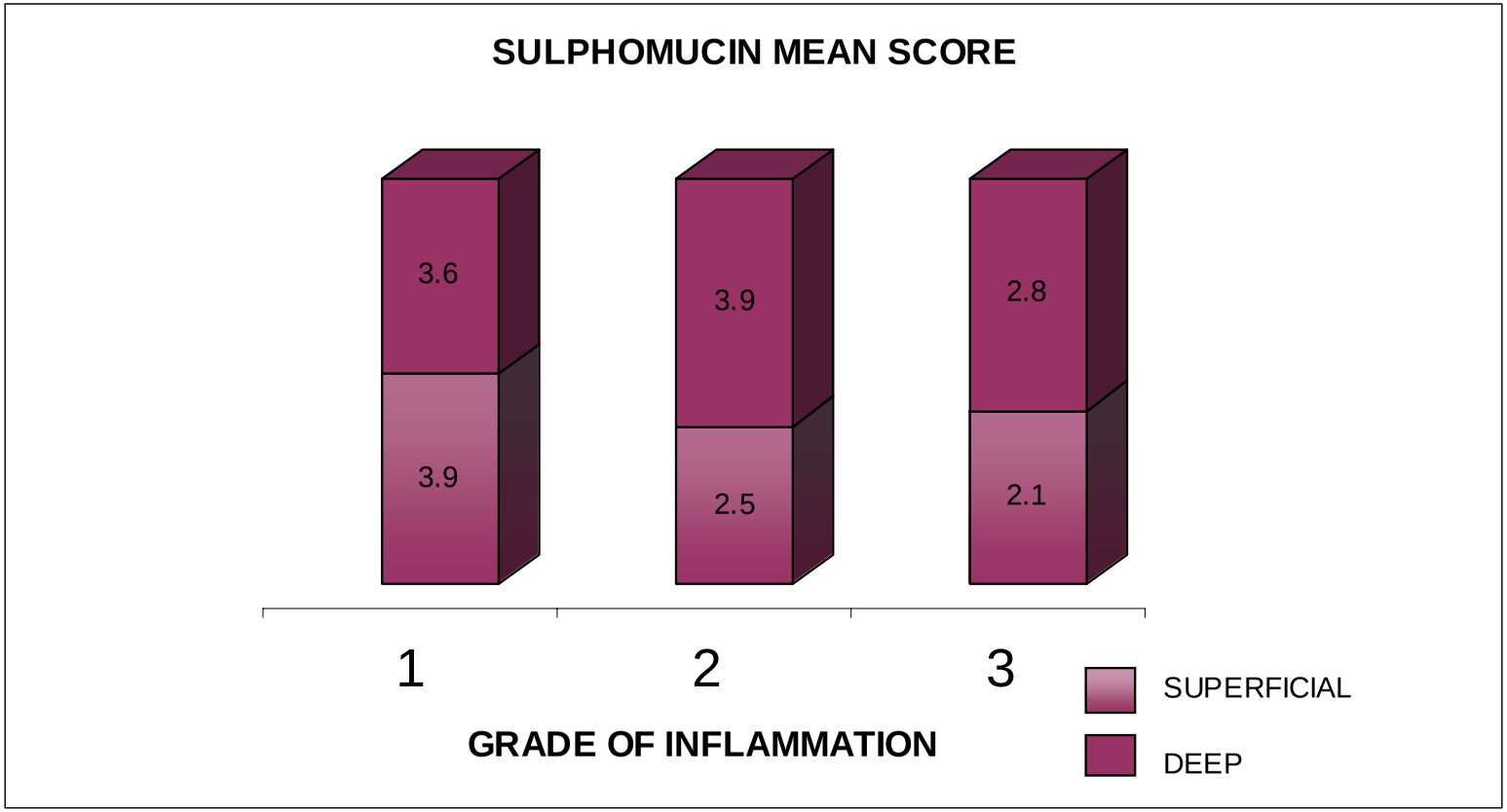
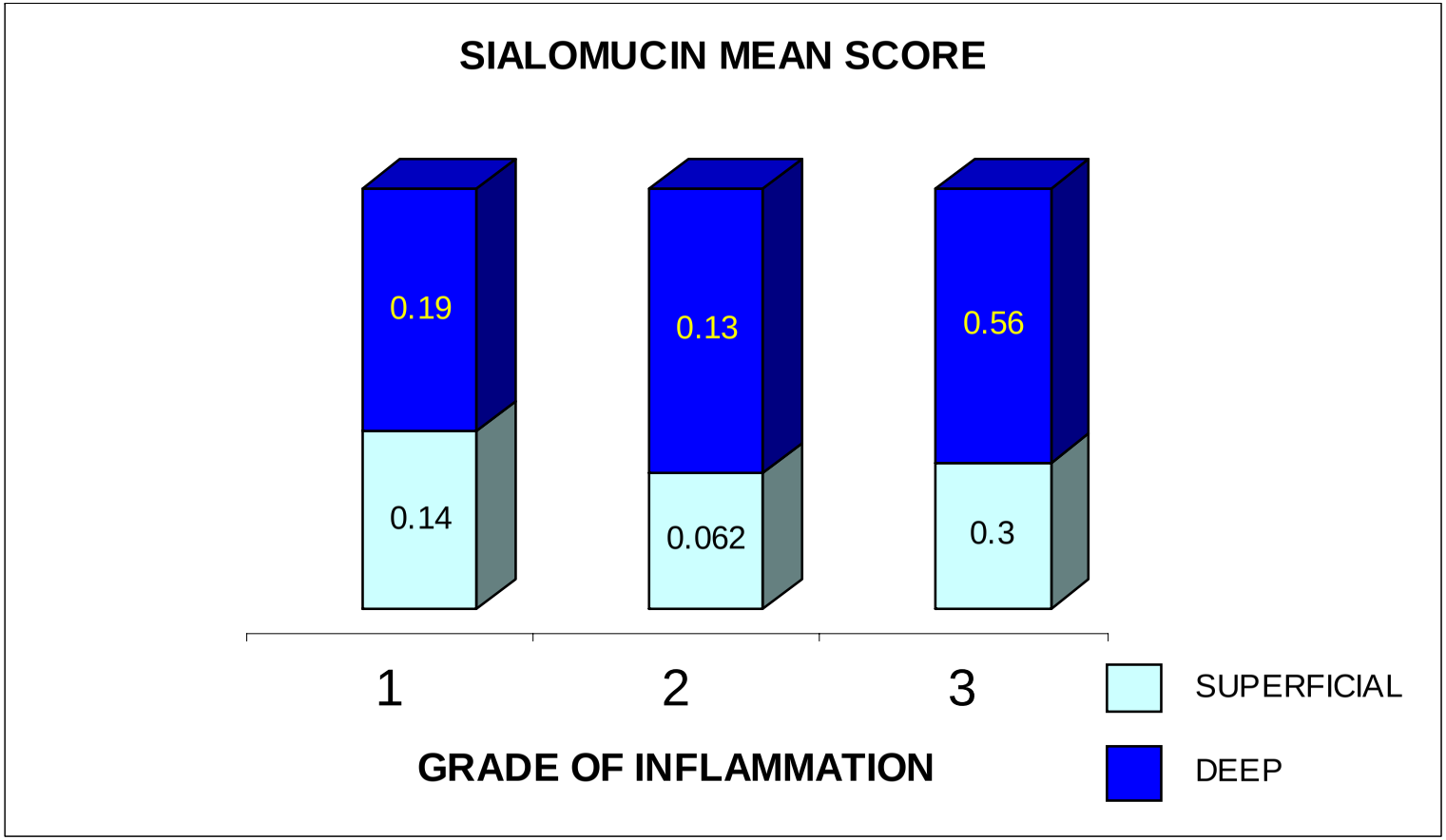

SULPHOMUCIN MEAN SCORE
3.6
3.9
3.9
2.5
2.8
2.1
1
2
3
GRADE OF INFLAMMATION
SUPERFICIAL
DEEP

TABLE 5

HID –AB STAIN

INFLAMMATION GRADE	ALCIAN BLUE – [SIALOMUCIN] MEAN SCORE	
	SUPERFICIAL	DEEP
I (9 cases)	0.14	0.19
II (16 cases)	0.062	0.13
III (11 cases)	0.3	0.56

Alcian Blue scores are tabulated against the three grades of inflammation. Here, unlike the HID scores, the highest values are seen in Grade III inflammation .

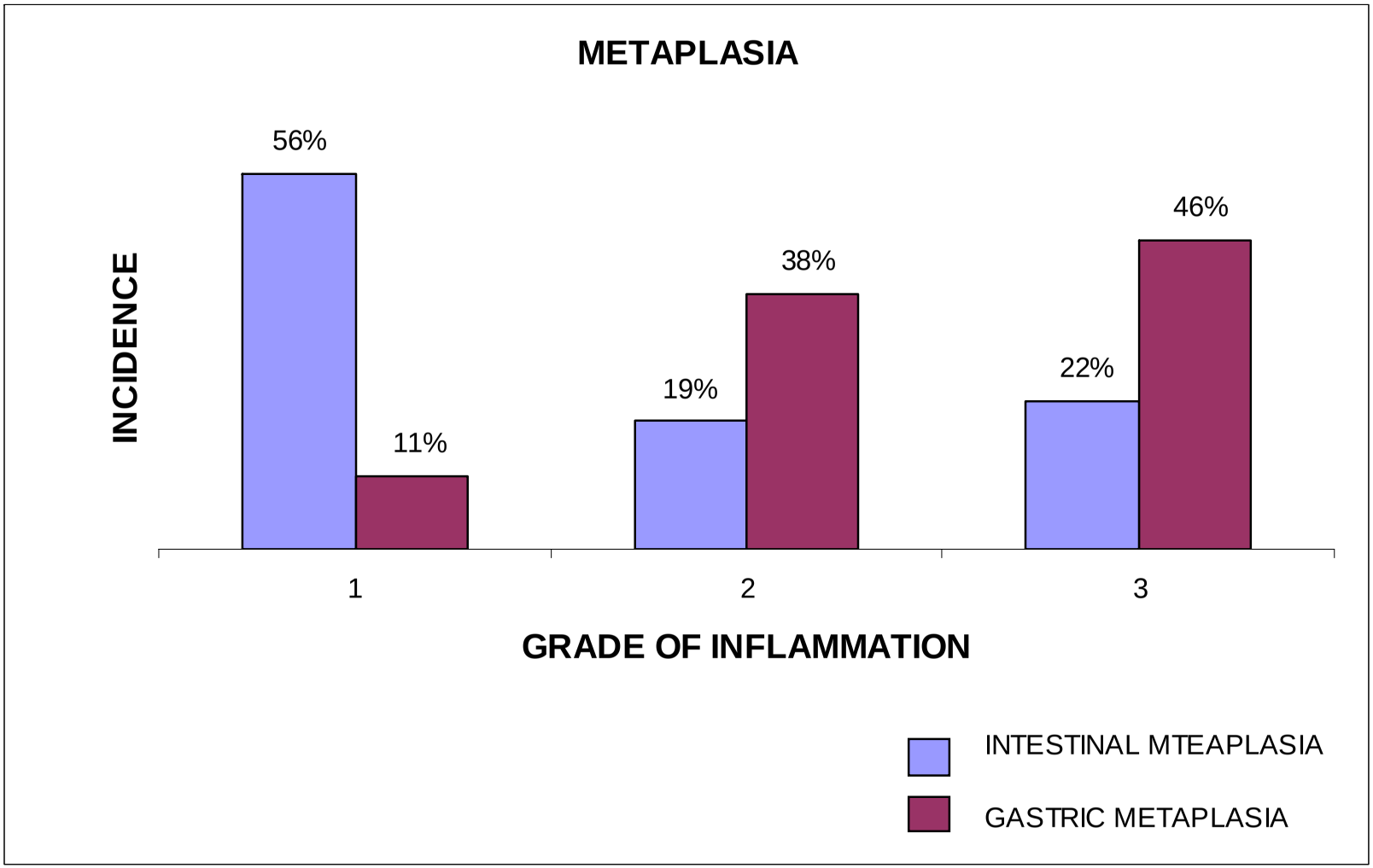

SIALOMUCIN MEAN SCORE
0.19
0.14
0.13
0.062
0.56
0.3
1
2
3
GRADE OF INFLAMMATION
SUPERFICIAL
DEEP

INFLAMMATION AND METAPLASIA

TABLE 6

TYPE OF METAPLASIA	INFLAMMATION GRADE			
	I (9 cases)	II (16 cases)	III (11 cases)	TOTAL 36
IM (INTESTINALMETAPLASIA)	5 (56%)	3 (19%)	5 (22%)	13
GM (GASTRIC METAPLASIA)	1 (11%)	6 (38%)	5 (46%)	12

The table shows the number of cases with intestinal and Gastric metaplasia and their percentage, in the three grades of inflammation. Metaplastic epithelium is characterized by presence of PAS positive mucins and / or goblets cells containing AB positive mucins in AB – PAS stains sections (figures 17,18,19,20,21). Intestinal metaplasia has the highest incidence in Grade I inflammation, and is lowest in Grade III inflammation .The reverse is seen with gastric metaplasia, which has the highest incidence in Grade III Inflammation, and a considerably lower incidence in Grade I inflammation .

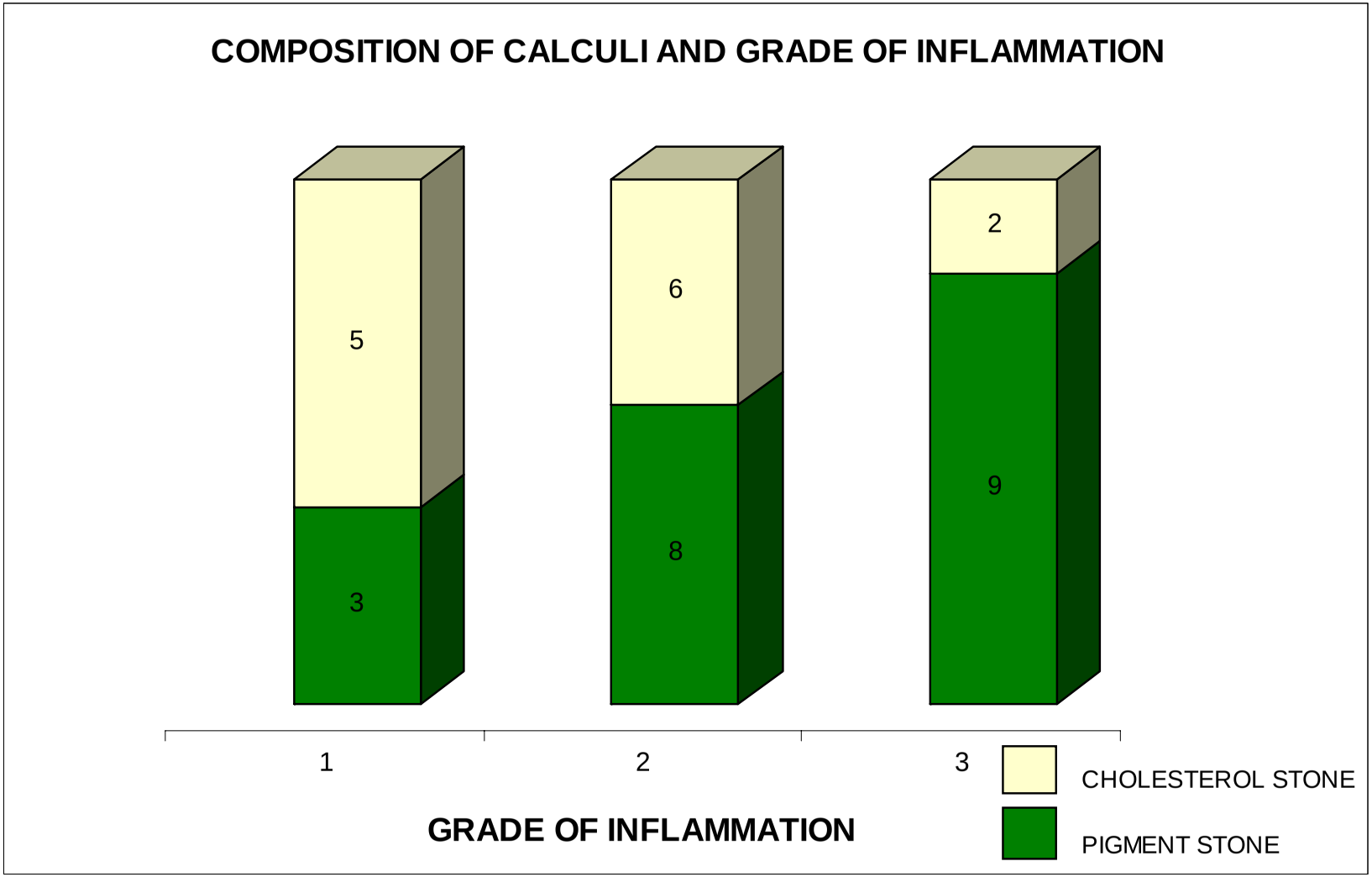

METAPLASIA
INCIDENCE
56%
11%
19%
38%
22%
46%
1
2
3
GRADE OF INFLAMMATION
INTESTINAL MTEAPLASIA
GASTRIC METAPLASIA

COMPOSITION OF CALCULI AND GRADE OF INFLAMMATION

TABLE 7

GRADE OF INFLAMMATION	PIGMENT STONE	CHOLESTEROL STONE
I (8 cases)	3	5
II (14 cases)	8	6
III (11 cases)	9	2
TOTAL - 33	TOTAL - 20	TOTAL - 13

Of the 36 cases with inflammation 3 did not have calculi suitable for biochemical analysis. The results tabulated in table VII are on the remaining 33 cases. There were 13 cases with cholesterol calculi and 20 with pigment calculi. Distribution of the two types of calculi among the three grades of inflammation is shown. The presence of pigment stones appears to correlate with severity of inflammation (3/8 in Grade I inflammation,9/11 in Grade III inflammation) as against cholesterol stones(5/8 in Grade I inflammation, 2/11in Grade III inflammation).


COMPOSITION OF CALCULI AND GRADE OF INFLAMMATION
5
3
6
8
2
9
1
2
3
GRADE OF INFLAMMATION
CHOLESTEROL STONE
PIGMENT STONE

COMPOSITION OF STONES AND MUCIN HISTOCHEMISTRY

TABLE 8

	PIGMENT STONES		CHOLESTEROL STONES	
TOTAL NUMBER	20		13	
SIALOMUCINS PRESENT IN	5		6	
SIALOMUCIN SCORE	SUPERFICIAL	DEEP	SUPERFICIAL	DEEP
	0.85	0.72	0.24	0.58

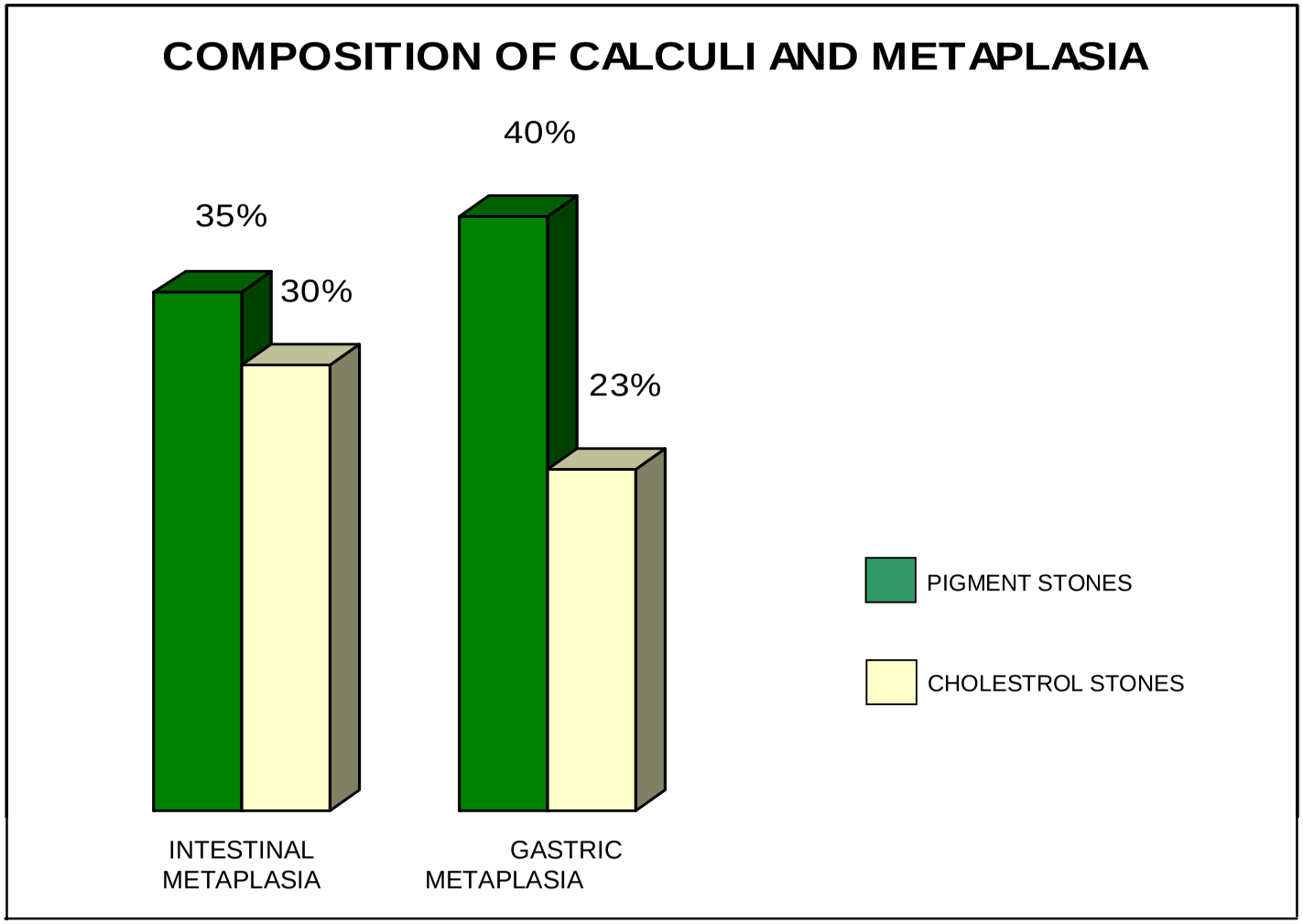
Mean scores of sialomucins in the superficial and deep mucosal epithelium are higher in cases with pigment stones (0.85,0.72),when compared with those having cholesterol stones (0.24,0.58)the number of cases that show presence of sialomucins is low with both types of calculi(5/20 cases with pigment stones and 6/13 cases with cholesterol stones).

COMPOSITION OF CALCULI AND METAPLASIA:

TABLE 9

TYPES OF METAPLASIA	PIGMENT STONES	CHOLESTEROL STONES
INTESTINAL METAPLASIA	35%	30%
GASTRIC METAPLASIA	40%	23%

It is shown that intestinal metaplasia is more or less equally associated with pigment and cholesterol calculi(35% and 30% incidence). Gastric metaplasia shows a considerably higher incidence (40%) in association with pigment stones, when compared with cholesterol stones (23%).


COMPOSITION OF CALCULI AND METAPLASIA
40%
35%
30%
23%
PIGMENT STONES
CHOLESTROL STONES
INTESTINAL METAPLASIA
GASTRIC METAPLASIA

FIBROSIS AND MUCIN HISTOCHEMISTRY

TABLE 10 & 11

AB- PAS STAIN

FIBROSIS GRADE	ALCIAN BLUE – [ACID MUCIN] MEAN SCORE		PAS – [NEUTRAL MUCIN] MEAN SCORE	
	SUPERFICIAL	DEEP	SUPERFICIAL	DEEP
I (19 cases)	2.46	2.81	0.13	0.22
II (9 cases)	3.0	3.1	0.96	0.45
III (5 cases)	2.0	1.7	0.85	0.32

Out of the 40 cases, 7 showed no fibrosis. The mean scores for remaining 33 cases are tabulated.

Alcian Blue scores (acid mucins) are slightly lower in Grade III fibrosis ,compared with Grade I . Neutral mucins (PAS positive) on the other hand shows higher scores in Grade III as against Grade I fibrosis.

TABLE 12

HID-AB STAIN

FIBROSIS GRADE	HID – [SULPHOMUCIN]		AB – [SIALO MUCIN]	
	SUPERFICIAL	DEEP	SUPERFICIAL	DEEP
I (19 cases)	2.64	3.52	0.11	0.17
II (9 cases)	3.08	3.27	0.26	1.97
III (5 cases)	1.7	3.7	0	0.08

No correlation is seen between HID scores (for sulphomucins) and degree for fibrosis. Alcian Blue scores (for sialomucins) are the lowest with Grade III fibrosis .

FIBROSIS AND STONE COMPOSITION

TABLE 13

GRADE OF FIBROSIS	PIGMENT STONE	CHOLESTEROL STONE	NO STONES	TOTAL
I	10	6	3	19
II	5	3	1	9
III	4	1	0	5
TOTAL (33)	23	13	4	33

Grade of fibrosis is correlated with type of calculus. Pigment stones have a higher incidence in Grade III fibrosis (4/5) as against Grade I fibrosis (10/19).

Statistically significant difference was seen in the sulphomucin content between Grade I and Grade III inflammation (Table 4). No statistical significance was detected in the other observations through application of tests like ANOVA, t-test, χ^2 (Chi^2) tests. However the total number of cases in each group was low, and more number of tissue samples needs to be studied to confirm the significance of these results. Rokitansky Aschoff sinuses showed no correlation with mucin histochemistry .

DISCUSSION

DISCUSSION

Attempts have been made in the past to correlate gallbladder morphology, mucin histochemistry, and composition of calculi in gallstone disease [16, 20, 39] .Most of the previous studies however have combined any two of the three aspects, that is, either morphology with mucin histochemistry ,mucin histochemistry with composition of calculi or composition of calculi with morphology. Few studies correlating all the three with one another have been recorded in literature .Purpose of the present study was to determine whether qualitative and / or quantitative variations in gallbladder mucins occurs in chronic calculous cholecystitis and whether the alterations ,if any, correlate with morphological changes in the gallbladder and / or with the type of calculous present .

In short, an attempt has been made through this study, to correlate gallbladder morphology, mucin histochemistry and composition of calculi to one another , in chronic calculous cholecystitis specimens.

Gallbladders removed for choledochal cysts were the control in the present study. The mucosal histology was normal and there was no inflammation, fibrosis or metaplasia. AB-PAS and HID-AB staining of sections showed predominantly HID positive sulphated acid mucins

throughout the mucosa,with traces of sialomucins and neutral mucins in foci.

Normal gallbladder mucosa is known to contain predominantly sulphomucins. Traces of sulphomucins and neutral mucins also may be present [39].

Our observations on control samples conform to this well – established normal pattern of mucin histochemistry of gallbladder mucosa.

Our results indicate a decrease in intraepithelial total acid mucin content in chronic calculous cholecystitis .Mucin depletion in mucosal epithelial cells is well known in inflammatory conditions of the gastrointestinal tract .In various forms of colitis presenting with mucus diarrhoea or dysentery and showing active inflammation of the mucosa ,mucin depletion is a constant finding . Increased mucin secretion by gallbladder mucosa during gallstone formation has been described in literature [6,7,8,9,39] .Earlier investigators have shown that mucins , in addition to being a structural component of gallstones [12], also play an acceleratory role in lithogenesis [29].

Our observation of decreased intraepithelial mucins in inflamed gallbladder mucosa is likely to be a reflection of increased secretion of mucin into bile which is known to occur in calculous disease.

The decrease in the intraepithelial mucins in chronic cholecystitis , we found , was due to decrease in sulphomucin which is the predominant type of mucin in gallbladder mucosa. Further, it was observed that cases with severe inflammation showed the maximum decrease in sulphomucin. This was associated with a concomitant increase in sialomucin scores and a high incidence of gastric metaplasia. Intestinal metaplasia on the other hand ,did not correlate with the degree of inflammation or sialomucin content .

No qualitative changes in gallbladder epithelial mucins have been observed in the earlier studies on chronic cholecystitis [37] . Sialomucins are known to occur in traces in normal gallbladder mucosa and in considerable quantities in metaplastic mucosa.

In the present study, an increase in sialomucins was observed in gallbladders showing severe inflammation .Interestingly, it was in this group that gastric metaplasia had the highest incidence (figure 22, 23).

It therefore follows that, sialomucins in significant quantities tend to appear in the areas of gastric metaplasia in the gallbladder mucosa. Their presence is not confined to the goblet cells of intestinal metaplasia which had the lowest percentage of incidence in severe inflammation, in the cases assessed figure (24,25).

It has been suggested that antral and intestinal metaplasia in the gallbladder are histogenetically related having the same progenitor cell ,and could therefore be parts of a morphological spectrum [44]. Transition from gastric to intestinal metaplasia is a likely possibility [17].

Pre - neoplastic role of intestinal metaplasia in the gallbladder and the metaplasia $\rightarrow$ dysplasia $\rightarrow$ neoplasia sequence have received wide attention among workers [11,17,61]. The mucin profile changes with progressive transformation to neoplasia, from normal with sulphomucin predominating through metaplastic and dysplastic showing increasing amounts of sialomucin, to full fledged neoplastic with sialomucins predominating .

The high incidence of gastric metaplasia in severe inflammation and its association with increased expression of sialomucins with the concomitant reduction in sulphomucins would point, perhaps tentatively,

towards a role for gastric metaplasia in the proposed chain of events stated above .

Basu et al studied the morphological changes in chronic calculous cholecystitis in relation to the type of stones [62]. They found that inflammation was more severe with pigment calculi while fibrosis and related complications were more frequent with cholesterol calculi.

The present study supports the association between pigment stones and severe inflammation. Fibrosis also was more in cases with pigment calculi in our study.

Mucins have been shown to be a structural component of gallstones[11,38]. Histochemical studies carried out on calculi have demonstrated presence of sulphomucins in them, especially in pigment stones [12].However no correlation between mucin histochemistry of mucosal epithelium and the type of stone has been recorded in literature.

In the present study, pigment stones were found more often in association with severe inflammation, gastric metaplasia and increased expression of sialomucins ,as against cholesterol stones.

We were unable to establish a statistical significance to the above observations, as the number of cases studied was small, especially in the sialomucin expressing group (even though the scores were high.)But the scores and percentage values did show a distinct pattern indicating a correlation between a severe inflammation, gastric metaplasia, sialomucins and pigment calculi.(Refer tables 4 to 9)

Considering the proposed pathway of Gastric metaplasia → Intestinal metaplasia → Dysplasia → Adenocarcinoma of gallbladder and the proven presence of sialomucins in considerable amounts in dysplastic and neoplastic gallbladder mucosa. It is reasonable to speculate on a pigment stone → severe inflammation → gastric metaplasia → sialomucin link up, with possible transition to dysplasia, with or without the intervention of intestinal metaplasia. Further studies on large series are required to enable us to draw definite conclusions. If such a high risk group emerges, it will be of significance from the preventive, prognostic and therapeutic point of view.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

I. The normal gallbladder epithelium contains sulphated acid mucins with traces of neutral and sialomucins .The sulphomucin content decreases in chronic calculus cholecystitis

II. In chronic calculus cholecystitis with severe (Grade III) inflammation (as against mild inflammation) :

1) Total acid mucin content is decreased.

2) This decrease is due to HID positive (sulpho) mucin.

3) Neutral mucin and sialomucin contents are increased.

4) There is a higher incidence of Gastric metaplasia and pigment stones .

III. Pigment gallstones tend to have an association with :

1) Severe inflammation

2) Higher degree of fibrosis

3) Gastric metaplasia

4) Presence of sialomucins

More number of cases need to be studied to see whether high risk group consisting of pigment stones → severe inflammation → gastric metaplasia → sialomucin emerges. If it does, will be of therapeutic and prognostic significance.

APPENDIX I

APPENDIX I

PROFORMA

Name: Hosp. No.

Age :

Sex :

Clinical Diagnosis:

Clinical History:

Radiological findings:

Operative findings:

PATHOLOGICAL FINDINGS

A.GROSS :

Size:

Contacted/distended/neither:

Wall thickness:

Outer surface:

Mucosal lining:

Calculi-single/multiple:

Colour/ type:

Adherent liver:

Lymph node;

Any other:

B. MICROSCOPIC :

a) Mucosa-

Epithelium:

Ulceration:

Inflammatory cells:

Foam cells:

Lymphoid follicles:

Epithelial regeneration:

Intestinal metaplasia:

Pyloric metaplasia:

Dysplasia:

b) Rokitansky Aschoff sinuses

c) Muscle coat :

Inflammation

Fibrosis

Abscess

Any other

d) Perimuscular tissue :

Inflammation

Fibrosis

e) Diverticulosis

f) Adherent liver

g) Lymph node

C. Mucin Histochemistry :

AB-PAS

HID-AB

D. BIOCHEMICAL ANALYSIS-RESULT

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BIBLIOGRAPHY

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MASTER CHART

MASTER CHART

INFLAMMATION - Grade 0

S.No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	2430/05	Sup. 3.7	Sup. 0	Sup. 2.7	Sup. 0	AB-PAS & HID-AB Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0	Deep 0	Deep 1.3	Deep 0		
2	103/06	Sup. 3	Sup. 0	Sup. 3	Sup. 0	AB-PAS Along the borders to 1/3rd of the cytoplasm HID-AB Along the borders	Biliary Sands
		Deep 2.8	Deep 0	Deep 4.8	Deep 0.1		
3	1798/06	Sup. 0	Sup. 0	Sup. 0	Sup. 0	AB 2/3rd of the cytoplasm HID 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.3	Deep 0	Deep 1.1	Deep 0		
4	84/07	Sup. 0.3	Sup. 0	Sup. 0.4	Sup. 0	AB 2/3rd of the cytoplasm HID Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.9	Deep 0	Deep 4.0	Deep 0		

INFLAMMATION - Grade 1

S. No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	2656/05	Sup. 3	Sup. 0	Sup. 4.8	Sup. 0	Sup. & Deep AB Along the borders Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0.7	Deep 0	Deep 0.7	Deep 0		
2	2663/05	Sup. 5	Sup. 0.1	Sup. 5	Sup. 0.2	Sup. & Deep AB-PAS & HID-AB Along the borders 1/3rd of the cytoplasm	Cholesterol Stones
		Deep 5	Deep 0.09	Deep 5	Deep 0.4		
3	133/06	Sup. 1.4	Sup. 0.29	Sup. 1.62	Sup. 0	Sup. & Deep AB-PAS & HID-AB Along the borders 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 3.5	Deep 0.7	Deep 4.25	Deep 0.5		
4	260/06	Sup. 1.6	Sup. 0	Sup. 3.6	Sup. 0.1	Sup. & Deep AB-PAS & HID-AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.9	Deep 0.1	Deep 4.8	Deep 0.2		
5	365/06	Sup. 4.3	Sup. 1.8	Sup. 5	Sup. 0.7	Sup. & Deep AB-PAS & HID-AB Along the borders, to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 4.3	Deep 0.3	Deep 5	Deep 0		
6	2674/06	Sup. 5	Sup. 00.7	Sup. 4.25	Sup. 0.3	Sup. & Deep AB-PAS & HID-AB Along the borders, to1/3rd of the cytoplasm	Biliary Sands
		Deep 0.3	Deep 0.1	Deep 0.04	Deep 0.6		
7	5/07	Sup. 3.5	Sup. 0	Sup. 4.3	Sup. 0	Sup. AB Seen along the borders, to1/3rd of the cytoplasm Sup. & Deep HID – AB 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.8	Deep 0	Deep 4.9	Deep 0		
8	161/07	Sup. 4.4	Sup. 0	Sup. 4.55	Sup. 0	Deep AB 2/3rd of the cytoplasm Sup. & Deep HID 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 5	Deep 0	Deep 4.61	Deep 0		
9	327/07	Sup. 1.3	Sup. 0	Sup. 1.9	Sup. 0	Sup. & Deep AB-PAS & HID-AB Along the borders, to1/3rd of the cytoplasm	Cholesterol Stones
		Deep 2.8	Deep 0	Deep 2.6	Deep 0		

INFLAMMATION Grade 2

S. No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	1282/05	Sup. 0.7 Deep 2	Sup. 0 Deep 0	Sup. 0.6 Deep 3	Sup. 0 Deep 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
2	2106/05	Sup. 1.6 Deep 4.9	Sup. 0.3 Deep 0	Sup. 1.6 Deep 5	Sup. 0 Deep 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID - AB Along the borders, to1/3rd of the cytoplasm	Cholesterol Stones
3	2529/05	Sup. 2.3 Deep 1.7	Sup. 0.5 Deep 0.8	Sup. 3.3 Deep 4.1	Sup. 0 Deep 0	Sup. & Deep AB - PAS Along the borders Sup. & Deep HID - AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
4	2570/05	Sup. 1.5 Deep 1.7	Sup. 0.2 Deep 0	Sup. 0.8 Deep 3.9	Sup. 0 Deep 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
5	2497/05	Sup. 0.3 Deep 1.2	Sup. 0 Deep 2.2	Sup. 0 Deep 0.9	Sup. 0 Deep 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Deep HID 1/3rd of the cytoplasm	Bile Pigment Stones
6	1212/06	Sup. 4.8 Deep 5	Sup. 0 Deep 0	Sup. 4.8 Deep 5	Sup. 0 Deep 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Deep HID Along the borders, to 1/3rd of the cytoplasm	Cholesterol Stones
7	1261/06	Sup. 3.8 Deep 3.8	Sup. 0 Deep 0.09	Sup. 5 Deep 4.75	Sup. 0 Deep 0	Sup. & Deep AB – PAS Along the borders to 2/3rd of the cytoplasm Sup. & Deep HID Along the borders to 2/3rd of the cytoplasm	Cholesterol Stones
8	1440/06	Sup. 1.1 Deep 4.1	Sup. 0 Deep 0.8	Sup. 2.5 Deep 4.1	Sup. 0 Deep 0	Sup. & Deep AB Along the borders to 2/3rd of the cytoplasm Deep HID 2/3rd of the cytoplasm	Biliary Sands

INFLAMMATION Grade 2 (Conti..)

S. No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
9	2069/06	Sup. 0.75	Sup. 0	Sup. 1	Sup. 0	Sup. & Deep AB 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.25	Deep 0.5	Deep 5	Deep 0	Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	
10	2119/06	Sup. 3.5	Sup. 0	Sup. 1.75	Sup. 0	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 0.4	Deep 0	Deep 3.5	Deep 0	Sup. & Deep HID 2/3rd of the cytoplasm	
11	2143/06	Sup. 1.5	Sup. 0	Sup. 3.8	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm	Biliary Sands
		Deep 2.3	Deep 0	Deep 3.4	Deep 0	Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	
12	2654/06	Sup. 4	Sup. 0	Sup. 4.4	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 5	Deep 0	Deep 4.7	Deep 0	Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	
13	2765/06	Sup. 2.2	Sup. 0	Sup. 3.5	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.7	Deep 0	Deep 5	Deep 0	Sup. & Deep HID Along the borders , to1/3rd of the cytoplasm	
14	2945/06	Sup. 3.6	Sup. 0	Sup. 3.25	Sup. 0.1	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 4.4	Deep 0	Deep 3.6	Deep 3.5	Deep HID – AB 1/3rd to 2/3rd of the cytoplasm	
15	3047/06	Sup. 1.1	Sup. 0.7	Sup. 2.35	Sup. 0	Sup. & Deep AB – PAS Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.8	Deep 0.5	Deep 4.8	Deep 0.5	Sup. & Deep HID Along the borders to 2/3rd of the cytoplasm	
16	222/07	Sup. 2	Sup. 0	Sup. 2.75	Sup. 0	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.6	Deep 0	Deep 4	Deep 0	Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	

INFLAMMATION - Grade 3

S. No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	1460/05	Sup. 4.3	Sup. 0	Sup. 4.3	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.1	Deep 0	Deep 3.4	Deep 0	Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	
2	1813/05	Sup. 1.58	Sup. 0	Sup. 1.5	Sup. 0	Sup. & Deep AB Along the borders	Cholesterol Stones
		Deep 0	Deep 0	Deep 1.16	Deep 0	Sup. & Deep HID Along the borders 1/3rd of the cytoplasm	
3	2216/05	Sup. 0.2	Sup. 1	Sup. 1.3	Sup. 0.2	Sup. & Deep AB-PAS 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 0.3	Deep 1.1	Deep 1.4	Deep 0.5	Sup. & Deep HID-AB 1/3rd to 2/3rd of the cytoplasm	
4	2634/05	Sup. 0	Sup. 0	Sup. 0	Sup. 0	Deep AB Along the borders	Bile Pigment Stones
		Deep 1	Deep 0	Deep 0.8	Deep 0	Deep HID Along the borders	
5	1031/06	Sup. 2.8	Sup. 0.3	Sup. 2.3	Sup. 1.5	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.7	Deep 0.4	Deep 0.3	Deep 0.7	Sup. & Deep HID-AB 1/3rd to 2/3rd of the cytoplasm	
6	1120/06	Sup. 2.68	Sup. 0.25	Sup. 3.4	Sup. 1.6	Sup. & Deep AB-PAS 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.3	Deep 0.74	Deep 3.6	Deep 1.1	Sup. & Deep HID-AB 1/3rd to 2/3rd of the cytoplasm	
7	1254/06	Sup. 5	Sup. 0	Sup. 2.6	Sup. 0	Sup. AB-PAS 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.25	Deep 0.6	Deep 5	Deep 0	Sup. HID-AB 2/3rd of the cytoplasm	
8	3145/06	Sup. 3.5	Sup. 1	Sup. 2	Sup. 0	Sup. & Deep AB-PAS 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 5	Deep 1.3	Deep 3.5	Deep 2.1	Sup. & Deep HID-AB 2/3rd of the cytoplasm	
9	3153/06	Sup. 0	Sup. 0	Sup. 1	Sup. 0	Deep AB-PAS Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0.4	Deep 0.5	Deep 4.2	Deep 0.4	Sup. & Deep HID-AB Along the borders to 1/3rd of the cytoplasm	
10	10/07	Sup. 0.7	Sup. 0	Sup. 2	Sup. 0	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 1.8	Deep 0.07	Deep 3.3	Deep 0	Sup. & Deep HID Along the borders	
11	66/07	Sup. 0.7	Sup. 0.1	Sup. 2.8	Sup. 0.07	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.8	Deep 0.2	Deep 3.6	Deep 0.07	Sup. & Deep HID-AB Along the borders to 1/3rd of the cytoplasm	

FIBROSIS - Grade 0

S.No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	1813/05	Sup. 1.58	Sup. 0	Sup. 1.5	Sup. 0	Sup. & Deep AB Along the borders Sup. & Deep HID Along the borders	Cholesterol Stones
		Deep 0	Deep 0	Deep 1.16	Deep 0		
2	2656/05	Sup. 3	Sup. 0	Sup. 4.8	Sup. 0	Sup. & Deep AB Along the borders Sup. & Deep HID Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0.7	Deep 0	Deep 0.7	Deep 0		
3	2663/05	Sup. 5	Sup. 0.1	Sup. 5	Sup. 0.2	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID-AB Along the borders to 1/3rd of the cytoplasm	Cholesterol Stones
		Deep 5	Deep 0.09	Deep 5	Deep 0.4		
4	133/06	Sup. 1.4	Sup. 0.29	Sup. 1.62	Sup. 0	Sup. AB-PAS 1/3rd to 2/3rd of the cytoplasm Sup. & Deep HID-AB 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 3.5	Deep 0.7	Deep 4.25	Deep 0.5		
5	2765/06	Sup. 2.2	Sup. 0	Sup. 3.5	Sup. 0	Sup. & Deep AB Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.7	Deep 0	Deep 5	Deep 0		
6	3145/06	Sup. 3.5	Sup. 1	Sup. 2	Sup. 0	Sup. & Deep AB-PAS 2/3rd of the cytoplasm Sup. & Deep HID-AB 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 5	Deep 1.3	Deep 3.5	Deep 2.1		
7	84/07	Sup. 0.3	Sup. 0	Sup. 0.4	Sup. 0	Deep AB 2/3rd of the cytoplasm Sup. & Deep HID-AB Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.9	Deep 0	Deep 4.0	Deep 0		

FIBROSIS - Grade 1

S.No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chem.
1	1282/05	Sup. 0.7	Sup. 0	Sup. 0.6	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2	Deep 0	Deep 3	Deep 0		
2	2106/05	Sup. 1.6	Sup. 0.3	Sup. 1.6	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID – AB Along the borders, to1/3rd of the cytoplasm	Cholesterol Stones
		Deep 4.9	Deep 0	Deep 5	Deep 0		
3	2216/05	Sup. 0.2	Sup. 1	Sup. 1.3	Sup. 0.2	Sup. & Deep AB - PAS Along the borders Sup. & Deep HID - AB Along the borders, to1/3rd of the cytoplasm	Cholesterol Stones
		Deep 0.3	Deep 1.1	Deep 1.4	Deep 0.5		
4	2570/05	Sup. 1.5	Sup. 0.2	Sup. 0.8	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 1.7	Deep 0	Deep 3.9	Deep 0		
5	2430/05	Sup. 3.7	Sup. 0	Sup. 2.7	Sup. 0	Sup. & Deep AB-PAS , HID-AB Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0	Deep 0	Deep 1.3	Deep 0		
6	103/06	Sup. 3	Sup. 0	Sup. 3	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID-AB Along the borders, to1/3rd of the cytoplasm	Biliary Sands
		Deep 2.8	Deep 0	Deep 4.8	Deep 0.1		
7	1120/06	Sup. 2.68	Sup. 0.25	Sup. 3.4	Sup. 1.6	Sup. & Deep AB-PAS , HID-AB 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.3	Deep 0.74	Deep 3.6	Deep 1.1		
8	1212/06	Sup. 4.8	Sup. 0	Sup. 4.8	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Deep HID Along the borders, to 1/3rd of the cytoplasm	Cholesterol Stones
		Deep 5	Deep 0	Deep 5	Deep 0		
9	1440/06	Sup. 1.1	Sup. 0	Sup. 2.5	Sup. 0	Sup. & Deep AB Along the borders to 2/3rd of the cytoplasm Deep HID 2/3rd of the cytoplasm	Biliary Sands
		Deep 4.1	Deep 0.8	Deep 4.1	Deep 0		

FIBROSIS - Grade 1 (Conti..)

S.No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chem.
10	1798/06	Sup. 0	Sup. 0	Sup. 0	Sup. 0	Deep AB 2/3rd of the cytoplasm Sup. & Deep HID 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.3	Deep 0	Deep 1.1	Deep 0		
11	2119/06	Sup. 3.5	Sup. 0	Sup. 1.75	Sup. 0	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm Sup. & Deep HID 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 0.4	Deep 0	Deep 3.5	Deep 0		
12	2654/06	Sup. 4	Sup. 0	Sup. 4.4	Sup. 0	Sup. & Deep AB Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID Along the borders, to1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 5	Deep 0	Deep 4.7	Deep 0		
13	2674/06	Sup. 5	Sup. 0.07	Sup. 4.25	Sup. 0.3	Sup. & Deep AB-PAS Along the borders, to1/3rd of the cytoplasm Sup. & Deep HID-AB Along the borders , to1/3rd of the cytoplasm	Biliary Sands
		Deep 0.3	Deep 0.1	Deep 0.4	Deep 0.6		
14	2945/06	Sup. 3.6	Sup. 0	Sup. 3.25	Sup. 0.1	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm Deep HID – AB 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 4.4	Deep 0	Deep 3.6	Deep 3.5		
15	3047/06	Sup. 1.1	Sup. 0.7	Sup. 2.35	Sup. 0	Sup. & Deep AB – PAS Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID Along the borders to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.8	Deep 0.5	Deep 4.8	Deep 0.5		
16	5/07	Sup. 3.5	Sup. 0	Sup. 4.3	Sup. 0	Sup. & Deep AB Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.8	Deep 0	Deep 4.9	Deep 0		
17	10/07	Sup. 0.7	Sup. 0	Sup. 2	Sup. 0	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 1.8	Deep 0.07	Deep 3.3	Deep 0		
18	161/07	Sup. 4.4	Sup. 0	Sup. 4.55	Sup. 0	Deep AB, 2/3rd of the cytoplasm Sup. & Deep HID 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 5	Deep 0	Deep 4.61	Deep 0		
19	222/07	Sup. 2	Sup. 0	Sup. 2.75	Sup. 0	Sup. & Deep AB 1/3rd to 2/3rd of the cytoplasm Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 2.6	Deep 0	Deep 4	Deep 0		

FIBROSIS - Grade 2

S. No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	2497/05	Sup. 0.3	Sup. 0	Sup. 0	Sup. 0	Sup. & Deep AB-PAS, HID-AB 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 1.2	Deep 2.2	Deep 0.9	Deep 0		
2	2529/05	Sup. 2.3	Sup. 0.5	Sup. 3.3	Sup. 0	Sup. & Deep AB-PAS, HID-AB Along the borders	Bile Pigment Stones
		Deep 1.7	Deep 0.8	Deep 4.1	Deep 0		
3	260/06	Sup. 1.6	Sup. 0	Sup. 3.6	Sup. 0.1	Sup. & Deep AB-PAS, HID-AB Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.9	Deep 0.1	Deep 4.8	Deep 0.2		
4	365/06	Sup. 4.3	Sup. 1.8	Sup. 5	Sup. 0.7	Sup. & Deep AB-PAS, HID-AB 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 4.8	Deep 0.3	Deep 5	Deep 0		
5	1031/06	Sup. 2.8	Sup. 0.3	Sup. 2.3	Sup. 1.5	Sup. & Deep AB-PAS Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID-AB 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.7	Deep 0.4	Deep 0.3	Deep 0.7		
6	1261/06	Sup. 3.8	Sup. 0	Sup. 0	Sup. 5	Sup. & Deep AB-PAS 2/3rd of the cytoplasm Sup. & Deep HID-AB 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 3.8	Deep 0.09	Deep 4.75	Deep 1		
7	2143/06	Sup. 1.5	Sup. 0	Sup. 3.8	Sup. 0	Sup. & Deep AB Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID Along the borders to 1/3rd of the cytoplasm	Biliary Sands
		Deep 2.3	Deep 0	Deep 3.4	Deep 0		
8	66/07	Sup. 0.7	Sup. 0.1	Sup. 2.8	Sup. 0.07	Sup. & Deep AB-PAS, HID-AB Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 3.8	Deep 0.2	Deep 3.6	Deep 0.07		
9	327/07	Sup. 1.3	Sup. 0	Sup. 1.9	Sup. 0	Sup. & Deep AB-PAS, HID-AB Along the borders to 1/3rd of the cytoplasm	Cholesterol Stones
		Deep 2.8	Deep 0	Deep 2.6	Deep 0		

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S.No	Biopsy No.	AB	PAS	HID	AB	Quantity	Bio – Chemistry
1	1460/05	Sup. 4.3	Sup. 0	Sup. 4.3	Sup. 0	Sup. & Deep AB Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 4.1	Deep 0	Deep 3.4	Deep 0		
2	2634/05	Sup. 0	Sup. 0	Sup. 0	Sup. 0	Deep AB Along the borders Deep HID Along the borders	Bile Pigment Stones
		Deep 0.75	Deep 0	Deep 1	Deep 0		
3	1254/05	Sup. 5	Sup. 4.25	Sup. 2.6	Sup. 0	Sup. AB-PAS 1/3rd to 2/3rd of the cytoplasm Sup. HID-AB 2/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0	Deep 0.6	Deep 5	Deep 0		
4	2069/05	Sup. 0.75	Sup. 0	Sup. 1	Sup. 0	Sup. & Deep AB 2/3rd of the cytoplasm Sup. & Deep HID 1/3rd to 2/3rd of the cytoplasm	Cholesterol Stones
		Deep 3.5	Deep 0.5	Deep 5	Deep 0		
5	3153/05	Sup. 0	Sup. 0	Sup. 1	Sup. 0	Deep AB-PAS Along the borders to 1/3rd of the cytoplasm Sup. & Deep HID-AB Along the borders to 1/3rd of the cytoplasm	Bile Pigment Stones
		Deep 0.4	Deep 0.5	Deep 4.2	Deep 0.4		